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Interlaboratory Comparison of a 28-Day
Toxicity Test with the Polychaete
'Neanthes arenaceodentata'

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Interlaboratory Comparison of a 28-Day Toxicity Test with the Polychaete *Neanthes* *arenaceodentata*

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ABSTRACT: An interlaboratory comparison of toxicity tests with the polychaete *Neanthes arenaceodentata* was conducted to determine the amount of variability expected with this test. Six laboratories each conducted two 28-day flow-through tests, one with silver [as silver nitrate (AgNO_3)] as the toxicant and the other with the pesticide endosulfan. Muffled sand (from nonpolluted beaches) was used as sediment in the tests. The mean 28-day median lethal concentrations (LC_{50} s) (based on measured concentrations) for the participating laboratories were $165 \pm 52 \mu\text{g/L}$ and $106 \pm 24 \mu\text{g/L}$ for the tests with silver and endosulfan, respectively. The ratio of the highest to the lowest LC_{50} value was 2.23 for silver and 1.81 for endosulfan. Therefore, the variability between laboratories was low for this test with *N. arenaceodentata*.

KEY WORDS: silver, toxicity test, pesticide, endosulfan, polychaetes, *Neanthes arenaceodentata*, interlaboratory comparison, aquatic toxicology, hazard assessment

Aquatic toxicity tests have been used in the past as management tools and are included in the Toxic Substances Control Act (TSCA) (P.L. 94-469) to regulate pollutants of the environment. However, one of the major concerns about aquatic toxicity tests is the reproducibility of the results. Before tests are used, especially as part of a regulatory process, the amount of variation expected in the results should be determined. Only a few aquatic toxicity test methods have been subjected to interlaboratory comparisons. Weiss and Helms [1]² con-

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²The italic numbers in brackets refer to the list of references appended to this paper.

ducted a freshwater algal assay comparison that involved eight laboratories. Davis and Hoos [2] presented the results of an interlaboratory comparison that used three species of freshwater fish, two toxicants, and seven laboratories. Reish et al [3] published the first interlaboratory comparison that used a saltwater organism, the polychaete *Capitella capitata*. Schimmel [4], Lemke [5], and Nebeker [6] have completed part of a series of interlaboratory comparisons with aquatic organisms that were planned specifically to respond to the need for standard methods for the TSCA.

The toxicity test with the polychaete (marine worm) *Neanthes (Nereis) arenaeodentata* Moore (*Nereis acuminata* Ehlers, *Nereis caudata* delle Chiaje) is also one of the tests being considered for use as a standard test for TSCA to measure the effect of chemicals on saltwater organisms. This test was included because *N. arenaeodentata* is a benthic organism and sediment was included in the test system. The purpose of this interlaboratory comparison was to determine the amount of variability in the data produced by different laboratories that followed the same test method. The test method specified a 28-day flow-through test with sediment, a clean sand. The 28-day median lethal concentration (LC_{50}) and median effective concentration (EC_{50}) (the concentration at which 50% of the test organisms were unable to burrow into the sediment) were calculated. The 28-day test period, rather than a 96 h test, was specified to provide time to see any interaction of toxicant with the sediment and to observe the sublethal parameter of inability to burrow. Six laboratories participated, two U.S. Environmental Protection Agency (EPA) Office of Research and Development laboratories and four contract laboratories. The two toxicants used were silver and endosulfan. These two chemicals were chosen because (1) they represent two chemical classes, organic and inorganic; (2) there are few published data on the toxicity of these chemicals to saltwater species; (3) they are relatively toxic to selected species; and (4) one of the chemicals, silver, required some experience to analyze it in salt water.

In this interlaboratory comparison, little effort was spent in attempting to isolate the possible sources of variation. Some of the sources of variation in toxicity tests are (1) population of the animals tested, (2) age or size of the animals, (3) the test method, (4) the food fed during acclimation and testing, (5) handling techniques, (6) the water quality, (7) the source of the toxicant, and (8) chemical analyses. Three of these eight sources of variation (Nos. 2, 3, and 7) were controlled in this interlaboratory comparison. The size of the test animals and the test method were specified. The participants were sent the toxicants (from the same source) to be used in the test. Some attempt was made to check variation due to chemical analyses. Quality control samples of dosed salt water from the experimental systems for each participating laboratory were sent to the lead laboratory (the EPA's Environmental Research Laboratory, Narragansett, RI) for chemical analysis. An attempt was made to minimize the variation due to food. The type of food to be fed to the test animals was specified, but each laboratory had to supply this food from its own source.

The only effort made to control handling techniques and the quality of the salt water was in the selection of the contract laboratories to participate in the study. A technical evaluation was made of each proposal submitted by a contract laboratory. Six laboratories submitted technically acceptable proposals. From these six laboratories, the four lowest bidders were selected. Four of the six participating laboratories had previous test experience with *N. arenaceodentata*. The other two laboratories had conducted flow-through toxicity tests with other saltwater species and had previous experience handling other polychaete species. All the laboratories had an adequate supply of "acceptable" salt water.

Materials and Methods

Toxicity Test

Each laboratory conducted two 28-day flow-through toxicity tests, one with silver and one with endosulfan, with the polychaete *N. arenaceodentata*. Two parameters were observed daily during the tests: inability of the worm to burrow into the sediment and death. In most cases the worms come to the surface of the sediment before they die.

Test Method

Participating laboratories were instructed to follow the ASTM "Proposed Standard Practice for Conducting Basic Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians" (Draft 7) and the contract Scope of Work.³ [ASTM has since published a final version of this as the ASTM Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians (E 729-80).] The ASTM document requires that certain conditions must be met ("must" statements) to produce satisfactory toxicity test data. Other conditions for good test practices are suggested ("should" statements) by ASTM but not required. The contract Scope of Work included some "must" statements not in ASTM Practice E 729-80 because the ASTM document was used only as a general guide for test procedures. The ASTM document does not include methods for longer tests (28 days), tests with sediment, or tests with *N. arenaceodentata*. The contract Scope of Work included "must" statements on (1) the salinity and temperature of the test water; (2) the size, feeding, and handling techniques for *N. arenaceodentata*; and (3) the type and handling of the sediment. These are mentioned in more detail in following appropriate sections. The final report from each laboratory was scrutinized to determine compliance with the "must" statements and the effect of deviations on the test results.

³Environmental Protection Agency Request for Proposal CI-79-0506.

Test Animals

The polychaetes used by all the laboratories were taken from laboratory cultures of *N. arenaceodentata*. Although not required, all the worms used in the tests originally came from a culture established by D. J. Reish at California State University, Long Beach, CA. Laboratories B, D, and F procured their test worms directly from Reish. Laboratory E used worms from Reish for the silver test but worms from their own established culture for the endosulfan test. Laboratories A and C procured their test animals from a commercial supplier (Sea Plantations,⁴ Salem, MA) who obtained their original stock from Reish. Young adult worms (1.5 to 2.5 cm in length) were used in the test. Females with eggs in their coeloms were not used.

Toxicant

Silver [as silver nitrate (AgNO_3), reagent grade, Allied Chemical, Morristown, NJ] and endosulfan (Thiodan, 94.4% pure, FMC Corp., Middleport, NY) were supplied to participating laboratories by us (Laboratory A). The carrier to be used for the endosulfan test was not specified; Laboratories A, B, C, and E used triethylene glycol, Laboratory F used acetone, and Laboratory D used methanol. The laboratories were required to measure the concentration of toxicant in each treatment a minimum of seven times during each test. The 28-day LC_{50} and EC_{50} values were calculated using the mean measured concentrations. To check variation in chemical analyses, participating laboratories sent samples taken during the first week and on the final day of the test from each treatment to the authors for chemical analysis. The analytical results from the two laboratories were compared.

Sediment

Because *N. arenaceodentata* is a benthic organism, sediment was used in the toxicity test. Since sediment type may affect the results of a toxicity test [7,8], all the laboratories were required to use sand collected from a nonpolluted area and baked in a muffle furnace (6 h at 500°C) to remove organic material. Laboratory C purchased sand from a commercial supplier, and the other laboratories collected sand from local nonpolluted beaches.

Food

The worms were fed *Enteromorpha* sp. three times weekly during the test. Laboratories B, D, and E obtained *Enteromorpha* from Reish, and the other laboratories supplied their own.

⁴Products or companies mentioned do not imply endorsement by the U.S. Environmental Protection Agency.

Dilution Water

All the tests were conducted with natural, filtered salt water with a salinity of 30 ± 2 g/kg and a temperature of $20 \pm 1^\circ\text{C}$.

Experimental System

All the tests were conducted in flow-through systems. ASTM Practice E 729-80 specifies conditions for the toxicant delivery system. The contract Scope of Work required that the worms be isolated during the test to prevent cannibalism.

Data Analysis

The LC_{50} values were calculated from nominal and mean measured concentrations. The EC_{50} values were calculated only from the mean measured concentrations. All the LC_{50} and EC_{50} values were calculated on a computer.⁵ This program calculates the results three ways—through probit analysis [9], the moving average method [10], and the binomial test [11,12].

Results

Eleven of the twelve toxicity tests required by this interlaboratory comparison were completed successfully (Table 1). There was good agreement in the 28-day LC_{50} values, based on nominal and measured concentrations, from the participating laboratories for the silver and endosulfan toxicity tests with *N. arenaceodentata*. The ratios of the highest LC_{50} value to the lowest, based on measured concentration, were 2.23 and 1.81 for the silver and endosulfan tests, respectively. The high/low ratios for the LC_{50} values, based on nominal concentrations, were a little higher, 3.25 for silver and 2.09 for endosulfan. Among the six laboratories, the LC_{50} values calculated from the measured concentrations were more consistent (a lower high/low ratio and smaller standard deviation) than the corresponding LC_{50} values calculated from the nominal concentrations. The LC_{50} values for the endosulfan test were less variable than those for the silver test.

Although the test method required only a 28-day LC_{50} value as the end point, the LC_{50} values for other days were also calculated because observations for mortality were made daily. The 96-h, 10-day, and 28-day LC_{50} values (based on measured concentrations) are compared in Tables 2 (silver) and 3 (endosulfan). Several "greater than" LC_{50} values are reported for 96-h and 10-day test periods because there were insufficient effects to calculate a value. These "greater than" values were used to calculate the mean, standard deviation.

⁵Charles Stephan, Probit Program, Environmental Research Laboratory, U.S. Environmental Protection Agency, Duluth, MN, personal communication, 1977.

TABLE 1—Twenty-eight-day LC_{50} values for the interlaboratory comparison with the polychaete *Neanthes arenaceodentata*.^a

Laboratory	Silver, $\mu\text{g/L}$		Endosulfan, $\mu\text{g/L}$	
	Nominal	Measured	Nominal	Measured
A	97 (82 to 116)	106 (90 to 124)	144 (120 to 174)	114 (93 to 141)
B	174 ^b (135 to 225)	126 ^b (115 to 139)	163 (144 to 187)	145 (127 to 167)
C	142 (123 to 166)	167 (145 to 195)	203 (173 to 244)	80 (63 to 104)
D	157 (122 to 204)	191 (156 to 237)	170 (147 to 192)	116 (90 to 140)
E	^d	^d	191 ^b (150 to 300)	89 ^b (72 to 134)
F	315 (275 to 363)	236 (211 to 267)	97 ^c (72 to 127)	95 ^c (72 to 122)
Mean	177	165	161	106
Standard deviation	82	52	37	24
High/low ratio	3.25	2.23	2.09	1.81

^aThe numbers in parentheses are 95% confidence limits. The LC_{50} values were calculated by moving average method unless otherwise noted.

^bThe number approximated by binomial method.

^cThe number calculated by probit method.

^dThe test was unacceptable (see text).

TABLE 2—Comparison of 96-h, 10-day, and 28-day LC_{50} and EC_{50} (inability to burrow) values for silver (measured concentrations, in micrograms per litre) for the interlaboratory comparison with the polychaete *Neanthes arenaceodentata*.^a

Laboratory	96 h		10 Days		28 Days	
	LC_{50}	EC_{50}	LC_{50}	EC_{50}	LC_{50}	EC_{50}
A	151	119	108	108	106	103
B	145	132	126 ^b	126 ^b	126 ^b	126 ^b
C	251 ^c	251 ^c	238 ^d	227 ^d	167	164
D	260	254	201	201	191	183
E	^e	^e	^e	^e	^e	^e
F	> 357 ^f	> 357 ^f	357	319	236	206
Mean	233 ^g	213 ^g 233 ^g	206	196	165	156
Standard deviation	88 ^g	98 ^g	100	85	52	42
High/low ratio	2.46 ^g	3.00 ^g	3.31	2.53	2.23	2.00

^a LC_{50} and EC_{50} values were calculated by the moving average method unless otherwise noted.

^bThe number approximated by binomial method.

^cFive-day value, 96-h data were not available because the test was not checked on weekends.

^dThe twelve-day value, ten-day data were not available because the test was not checked on weekends.

^eThe test was unacceptable (see text).

^fThe value is expressed as "greater than" because there were insufficient effects to calculate an LC_{50} or EC_{50} .

^gThe "greater than" LC_{50} and EC_{50} values were used to calculate these numbers, therefore, these values represent the lower limit.

TABLE 3—Comparison of 96-h, 10-day, and 28-day LC_{50} and EC_{50} (inability to burrow) values for endosulfan (measured concentrations, in micrograms per litre) for the interlaboratory comparison with the polychaete *Neanthes arenaceodentata*.^a

Laboratory	96 h		10 Days		28 Days	
	LC_{50}	EC_{50}	LC_{50}	EC_{50}	LC_{50}	EC_{50}
A	220 ^b	195 ^b	114 ^b	114 ^b	114	114
B	> 243 ^c	> 243 ^c	> 243 ^c	> 243 ^c	145	145
C	190 ^{bd}	169 ^{bd}	169 ^b	169 ^b	80	77
D	161	150	132	128	116	116
E	> 134 ^c	> 134 ^c	> 134 ^c	> 134 ^c	89 ^b	89 ^b
F	222	186	158	158	95 ^e	92 ^e
Mean	195 ^f	180 ^f	158 ^f	158 ^f	106	105
Standard deviation	41 ^f	38 ^f	46 ^f	46 ^f	24	25
High/low ratio	1.18 ^f	1.81 ^f	2.13 ^f	2.13 ^f	1.81	1.88

^a LC_{50} and EC_{50} values were calculated by the moving average method unless otherwise noted.^bThe number approximated by binomial method.^cValue is expressed as "greater than" because there were insufficient effects to calculate an LC_{50} and EC_{50} value.^dThe six-day value, 96-h data were not available because the test was not checked on weekends.^eCalculated by probit method.^fThe "greater than" LC_{50} and EC_{50} values were used to calculate these numbers; therefore, these values represent the lower limit.

and high/low ratio; therefore, these statistics (for 96-h silver test and 96-h and 10-day endosulfan test) represent the lower limit. The mean silver LC_{50} values (based on measured concentrations) for 96 h, 10 days, and 28 days were 233, 206, and 165 $\mu\text{g/L}$, respectively. The 96-h and 10-day LC_{50} values for the silver test are not as consistent as the 28-day value as indicated by the high/low ratio. The mean endosulfan LC_{50} values (based on measured concentrations) for 96 h, 10 days, and 28 days were 195, 158, and 106 $\mu\text{g/L}$, respectively. The high/low LC_{50} ratio for the endosulfan test was about 2 for all three time periods. The 28-day LC_{50} values were within a factor of 2 of the 96-h LC_{50} values for these toxicants.

The other parameter observed during the toxicity tests was inability of the worm to burrow into the sediment. The concentration at which 50% of the worms were affected (dead worms plus live worms unable to burrow), the EC_{50} , was calculated for 96 h, 10 days, and 28 days (Tables 2 and 3). The mean of the EC_{50} values is slightly lower but not statistically different from the corresponding LC_{50} value for both the silver and endosulfan tests because most of the live worms were able to burrow.

The results of the quality control samples with silver varied. Comparisons were made between each laboratory's own measured silver concentration and the authors' analysis of the sample. Each laboratory was rated as follows: a dif-

ference of $\leq 10\%$ was rated as excellent; $\leq 15\%$ was good; $\leq 20\%$ was fair; $\leq 25\%$ was acceptable; and $> 25\%$ was unacceptable. There was good agreement for the silver analyses for two of the laboratories. Laboratory F had only one analysis out of twelve that was unacceptable; 92% of their analyses were acceptable, with 58% rated as having excellent agreement. Laboratory C had 64% of its analyses acceptable with 43% rated as excellent. Most of Laboratory C's unacceptable analyses were from the second sampling day. These unacceptable samples were from the control, the solvent (deionized water) control, and the two lowest treatments and would not have affected their 28-day LC_{50} value. Sampling time may account for the differences. Laboratory C did not use split samples for the quality control samples on this day, so the samples analyzed by us were taken on the same day but not at the same time. Therefore, this was not really a good quality control check. The differences in these analyses could be due to sample difference rather than an error in analytical method. The other two laboratories did not have good agreement on their quality control samples; only 29% and 20%, respectively, of Laboratories B and D's samples were rated acceptable.

Laboratory B had problems with its analytical method. Its analyses, 30 and 0 $\mu\text{g/L}$ of silver, of a spiked sample, 48 $\mu\text{g/L}$ of silver, indicated that Laboratory B had problems in analyzing for silver in salt water. Its analytical results for the unacceptable samples were about 50% lower than our results. However, Laboratory B's 28-day LC_{50} value would still have been lower than the highest LC_{50} value (reported by Laboratory F). Laboratory D also had problems with its analytical method for silver. The method it used had a detection limit of about 50 $\mu\text{g/L}$ of silver (Hoffman, unpublished results); therefore, the concentrations reported in control samples (30 and 50 $\mu\text{g/L}$ silver) were not real. Laboratory D incorrectly interpreted background noise as a reading. As a result, all of its reported analyses are higher (by varying percentages) than the actual amount of silver present. However, Laboratory D's 28-day LC_{50} value would not have been any lower than the lowest LC_{50} value (reported by Laboratory A). There were no quality control samples from Laboratory E because of a communication problem.

There were quality control samples for endosulfan from only one laboratory because the extracted samples from the other laboratories leaked from the vials during shipment. The results of this one set of samples from Laboratory F were excellent; only one analysis out of eleven was considered different.

The final reports were scrutinized to determine if the "must" statements of ASTM Practice E 729-80 and the contract Scope of Work had been fulfilled. These "must" statements specified certain conditions to ensure the quality of (1) the salt water, (2) the test animals, (3) the test procedures, (4) the test apparatus, and (5) the chemistry requirements. A detailed account of compliance with or deviation from the "must" statements, and also the results of the quality control samples (reported earlier) were included in the authors' report [13] to the EPA Office of Pesticides and Toxic Substances. Some of the laboratories

deviated from the "must" statements, but only those deviations that may have affected the results of the test are discussed here.

ASTM Practice E 729-80 specifies that the control mortality in acute toxicity tests must be $\leq 10\%$. All but one test satisfied this requirement. Laboratory E reported control mortality of 15% in its silver test. This mortality may have been due in part to a failure to isolate the worms. The *N. arenaceodentata* must be isolated to ensure that all deaths result from toxic effects and not from cannibalism. Laboratory E reported missing worms as dead. These missing worms cannot be counted as dead from the toxic effects of silver. If the missing worms are omitted, then the mortality in Laboratory E's highest concentration was only 42%, and an LC_{50} value for silver cannot be calculated. For this and other reasons listed later, Laboratory E's silver test was considered unacceptable. The missing worms were omitted from the calculation of Laboratory E's LC_{50} value for the endosulfan test. The control mortality for Laboratory E's endosulfan test was acceptable.

The ASTM Standard Procedure (Draft 7) required that the measured concentration of toxicant in any exposure chamber must not be any more than 30% higher or lower than the calculated (nominal) concentration. The final ASTM version (ASTM Practice E 729-80), however, just suggests that a difference of greater than 30% indicates a problem with the metering system or the properties of the toxicant. Laboratory A was the only laboratory to meet this requirement for all concentrations in both tests. Laboratories B, C, D, and F had a few treatments in one or both tests in which the measured concentration was greater than 30% different from the nominal concentration. Laboratory E did not meet this requirement for any of the concentrations. Its measured concentrations for the silver test were all more than 30% lower than the nominal concentrations and were also very inconsistent. On two occasions, no silver was detected in the highest treatment. The deviations from this ASTM requirement indicate that a number of the laboratories had problems with their dosing systems, particularly in the silver test, even though all the laboratories had acceptable testing apparatus according to ASTM specifications. Laboratory E had the greatest problem, and its silver test was the only test that was considered unacceptable.

Most of the laboratories met all of the analytical chemistry requirements in the ASTM Proposed Standard Procedures and the contract Scope of Work. The ASTM document (Draft 7 and ASTM Practice E 729-80) requires validation of the analytical method by analyzing for a known addition at the same concentration as the next-to-the-lowest toxicant concentration to be used in the test. All of the laboratories claimed that they validated their analytical method with a sample of known addition, but only Laboratory B listed these results in its final report. Even though all the laboratories claimed to have validated their methods, the analyses of split samples by Laboratories B and D did not agree with our analyses of these quality control samples (discussed earlier).

Discussion

The results of this interlaboratory comparison with *N. arenaceodentata* produced good agreement between laboratories for both toxicants. The LC_{50} values for 96 h and 10 days for the silver test were not as consistent between the laboratories as the 28-day LC_{50} values. The 28-day LC_{50} values were within a factor of 2 of the 96-h values for these two toxicants. If this test with *N. arenaceodentata* was going to be used to assess only the acute response of the worm to a toxicant, a 96-h test period would be sufficient. However, if a toxicant does interact with the sediment, a longer test period of 10 or 28 days may provide time to see this interaction. The advantage of using *N. arenaceodentata* as a toxicity test organism is that it is a benthic animal. This test system represents an opportunity to assess the interaction of the toxicant, animal, and sediment and to measure a sublethal parameter—burrowing. There is a need for a "benthic assay" to test the effect of contaminated sediment on a benthic organism. The authors plan to adapt this test using *N. arenaceodentata* to assess the effects of contaminated sediments.

The EC_{50} values for these two toxicants did not differ much from the corresponding LC_{50} values because the sublethal concentrations used in these tests did not adversely affect burrowing. Inability to burrow is an important behavioral response for a polychaete because, in the natural environment, the worm has no other protection from predators. Since this is an easy and inexpensive parameter to measure and is potentially important, it is recommended that the EC_{50} as well as the LC_{50} values be measured for toxicity tests with *N. arenaceodentata*.

The choice of sediment type may affect the results of a toxicity test [7,8]. Some toxicants, for example, copper, interact with organic material in the sediment and, thus, effectively lower the acute toxicity to the test organism. Therefore, the authors chose a sediment with minimal organic content to use in this interlaboratory comparison. If there is any interaction between the toxicant and organic material, this toxicity test provides the worst-case situation for a short-term test. A toxicity test conducted with sediment of a higher organic content and a toxicant that interacted with the organic material would produce a higher LC_{50} value for a short-term test [8].

The good agreement of the LC_{50} values indicates that there were no major problems with the test method or the biological aspects of this toxicity tests. Comparison of the quality control samples indicates that some of the laboratories had problems with the chemical analyses and their dosing systems in the test with silver. In spite of the problems with the chemical analyses, there was still good agreement in LC_{50} values because the biological and other sources of variability included in this interlaboratory comparison were greater than the chemical variability. The lowest and highest LC_{50} values for silver were reported by laboratories that had no problems with the chemical analyses. Laboratory

C's unacceptable analyses were for samples from control and low treatments and would not have affected the laboratory's 28-day LC₅₀ value. Analyses by Laboratory B and D were lower and higher, respectively, than the authors'; but the percentage error in their chemical analyses would still have kept their LC₅₀ values within the range of the highest and lowest values reported by Laboratory F and A, respectively.

Silver was chosen as one of the toxicants because it is difficult to analyze and use in a toxicant dosing system. Two of the laboratories that participated had problems with the chemical analyses of silver, and one laboratory did not complete an acceptable test with silver. These results emphasize the need to have a good analytical chemist to work with the biologists who conduct toxicity tests. Endosulfan is a relatively easy organic chemical to analyze and use in a toxicant dosing system, and the participating laboratories did not have problems with this chemical. The purpose of an interlaboratory comparison can be altered by the selection of the toxicant. If the purpose is to test the variation in all areas, biological and chemical, then a toxicant that poses some problems, such as silver, would provide a good test. If the purpose is just to test the biological variation, then the toxicant chosen should be one that is relatively easy to analyze, or an analytical chemistry comparison could be conducted prior to the biological tests.

In conclusion, the variability of the toxicity test with the polychaete *N. arenaceodentata* for 28 days was about a factor of 2. There were no major problems with the test method or the biological aspects of the test. The details that caused some problems for participating laboratories were the chemical analysis of silver and the maintenance of the proper test concentrations, particularly of silver, in the experimental systems.

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